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Christoffer Bro

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BROWDY AND NEIMARK, P.L.L.C.
624 NINTH STREET, NW
SUITE 300
WASHINGTON, DC 20001-5303

EXAMINER

LEAVITT, MARIA GOMEZ

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1633

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/536,495	Applicant(s) BRO ET AL.	
	Examiner MARIA LEAVITT	Art Unit 1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 09 November 2009.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1 and 11-19 is/are pending in the application.
- 4a) Of the above claim(s) 14-16 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1, 11-13, 17-19 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

Detailed Action

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
2. Status of claims. Claims 1 and 11-19 are pending. Claim 19 has been added by Applicant's amendment filed on 01-12-2009. Please, note that the claim listing provided and filed on 01-12-2009 does not amend or change claims 1 and 11-18. Claims 14-16 were previously withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 12-13-2007.
3. Therefore, claims 1, 11-13 and 17-19 are pending for examination to which the following grounds of rejection are applicable.

Nature of the invention

The present invention is drawn to a metabolically engineered *S. cerevisiae* expressing a heterologous recombinant *Streptococcus mutants* GapN gene on a multicopy plasmid which encodes for the **non-phosphorylating NADP⁺- dependent glyceraldehyd-3-phosphate dehydrogenase (GAPN or GAPDHN)** (p.11 lines 29-32). GAPN catalyses the irreversible oxidation of glyceraldehyde-3-phosphate and NADP⁺ into 3-phosphoglycerate and NADPH and does not use Pi as a substrate. Thus the reaction catalyzed by GAPN or GAPDHN yields one NADPH with not net energy yield, e.g., ATP. In contrast, the glycolytic reaction catalyzed by **NAD⁺-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH)** and subsequent reaction catalyzed by phosphoglycerate kinase (PGK or pgk) yields one NADH and one ATP (p.

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10, lines 14-32). Additionally, the specification discloses that a major problem in connection with ethanol production by anaerobic fermentation of *S. cerevisiae* is a substantial formation of glycerol as a by-product with loss of carbon in the bio-product (e.g., ethanol, amino acid, antibiotics) (p. 2, lines 14-16). Moreover, the specification discloses that during “anaerobic growth of *S. cerevisiae* on a fermentable sugar, surplus amounts of NADH are formed which cannot be used in generation of ATP, and this results in the formation of by-products, primarily glycerol” (p. 4, lines 14-19). It is well known in the art that under anaerobic conditions cytosolic NADH formed from biomass formation can only be reconverted to NAD⁺ via glycerol formation (p. 2, lines 19-24). “Glycerol is formed by *S. cerevisiae* during anaerobic growth to maintain the cytosolic redox balance. Thus, under anaerobic conditions NADH, produced as the result of production of biomass and organic acids, can only be oxidised to NAD⁺ by formation of glycerol, since respiration is not possible and the formation of ethanol is a redox-neutral process. The formation of glycerol is therefore a redox problem, so by introducing gapN into *S. cerevisiae*” the following metabolic modification are observed: the production of glycerol is reduced by one molecule for each molecule of glyceraldehyde-3-phosphate that is converted via GAPN and the flux redirected to ethanol and/or biomass thereby increasing the ethanol yield (p. 12, lines 19-32).

Rejections maintained in response to Applicants' arguments or amendments

Claim Rejections - 35 USC § 103

Claims 1, 11-13, 17 and 18 remain rejected and claim 19 is newly rejected under 435 U.S.C. 103(a) as being unpatentable over of Nissen et al., (Metabolic Engineering, 2000, 2: pages 69-77) in view of Valverde et al., (FEBS, 1999, 21898, pages 153-158).

Response to Applicants' Arguments as they apply to rejection of claims 1, 11-13 and 17-19 under 35 USC § 103

At pages 4-9 of the remarks filed on 11-09-2009, Applicants essentially argue that: 1) Nissen et al., does not disclose reducing formation of NADH and ATP by the enzymatic activity of a non-phosphorylating dehydrogenase (e.g. GAPN aka GAPDHN), 2) Nissen et al., does not disclose reducing formation of NADH at all, when Nissen et al., speaks of reduced formation of surplus NADH, this is not in fact via the mechanism of reduction of NADH formation, but via provision of a pathway for consuming NADH, 3) Nissen et al., teachings are significantly misstated by the Examiner, particularly because Nissen does teach reduction in the production of NADH but rather provision of an NADH consuming path that does not lead to glycerol, 4) distinct mechanisms for reducing surplus NADH undermines the Examiner's arguments, which starts from a false premise". The above arguments have been fully considered but deemed unpersuasive.

Regarding 1), 2), 3), and 4), the fact that Nissen et al., teaches a metabolically engineered *S. cerevisiae* for enhanced production of ethanol by reduced formation of surplus NADH and increased consumption of ATP rather than decreased formation of NADH, as Applicants content, is not disputed. However, two or more metabolically engineered pathways in anaerobic fermentation can lead to the same goal of redirecting the flux of carbon from glycerol toward ethanol. Indeed, Applicants clearly illustrates at pages 6 and 7 of the remarks filed on 11-09-2009 the specific net stoichiometry of two

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distinct metabolic routes to redirect the flux of carbon from glycerol toward ethanol (i) by modifying the ammonia assimilation pathway, as taught by Nissen, and (ii) by transforming a glucose -metabolizing *S. cerevisiae* with a recombinant non-phosphorylating NADP⁺-dependent GAPDH, encoded by the *gapN* gene, as exemplified in Examples 2 and 3 of the specification as filed. While the two metabolically engineered pathways e.g., (i) and (ii) as strategies for the redirection of flux of carbon from glycerol toward ethanol encompass different metabolic fermentative reactions, both pathways contribute to the redox balance in the total metabolism of sugars by yeast. One of ordinary skill in the art can appreciate that, under anaerobic conditions, engineering of different metabolic pathways involving the co-factors NADH and/or NADPH can lead to the regulation of the redox metabolism in glucose -metabolizing *S. cerevisiae* and improvement of intracellular cofactor concentrations in *S. cerevisiae*.

At pages 7, 8 and 9, Applicants reiterates the arguments previously presented at pages 17 and 18 of Applicants' remarks filed on 06-09-2008, essentially contending that: 1) Nissen discloses at page 70, col. 1, a problem encountered with a previous strategy to avoid the production of glycerol wherein the genes encoding GPD1 and GPD2 (e.g., isoenzymes of glycerol-3-phosphate dehydrogenase) which led to a transformed yeast unable to grow under anaerobic conditions due to intracellular accumulation of NADH "since under there conditions no alternative pathways exists in *S. cerevisiae* in which NADH is reoxidized to NAD⁺" (Nissen, page 70, col. 1, paragraph 2); 2) "the skilled artisan reading Nissen et al., would be discouraged by Nissen et al., from adopting any steps that would result in failure to grow under anaerobic conditions" , 3) Valverde et al., discloses a metabolically engineered *E. coli* in which the NAD-dependent glycolytic

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phosphorylating G3P dehydrogenase GAPDH was deleted and in which GAPN was expressed resulting in the decreased growth under anaerobic conditions, 4) it would not have been obvious to modify Nissen's yeast to include Valverde's GAPN-mediated pathway, thus a skilled artisan would therefore not perceive Valverde et al., as offering a teaching likely to be useful in yeast as an alternative strategy for obtaining the objects of Nissen et al., (reduced glycerol and increased ethanol"), and 5) In response to the Examiner's arguments set forth at pages 12-15 of the office action filed on 05-11-2009, Applicants at page 8 of the remarks of 11-09-2009 further argue that "Valverde does not teach different metabolic requirements which would fully overcome the problem of reduced growth. Hence, the Examiner's burden is to demonstrate that it would have been obvious how to overcome this problem and thus this negative teaching inherent to Valverde". The above arguments have been fully considered but deemed unpersuasive.

Regarding 1), 2), 3), 4) and 5), the examiner refers Applicants to the reasons already of record as set forth at pages 12-15 of the office action filed on 05-11-2009. Applicants appear to essentially argue that because the recombinant *E. coli* gap mutant W3CG with a mutated inactive GAPDH (phosphorylating NAD⁺-dependent glyceraldehyde-3-phosphate dehydrogenase, GAPDH), transformed with an expression vector encoding and expressing a functional plant GAPDHN (non-phosphorylating NADP⁺-dependent glyceraldehyed-3-phosphate dehydrogenase), as taught by Valverde is unable to grow under anaerobic conditions, it would have been unobvious to modify Nissen's yeast growing under anaerobic conditions to include Valverde's GAPN-mediated pathway which leads to reduced growth of the *E. coli* W3CG GAPDHN. At the outset, the Examiner strenuously disagrees with Applicants' position that it would have

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been unobvious to a skilled artisan to arrive at the instant invention through the combination of Nissen in view of Valverde. Indeed, as stated by the Examiner at page 11 of the office action of 05-11-2009, expression of a functional GAPDHN in a W3CG mutant is not the sole variable determining growth of *E. Coli* during fermentation, as partial growth of a W3CG mutant with only GAPDHN is observed in M63 medium supplemented with succinate plus glycerol (page 157, Fig. 3). Most important, W3CG mutant with only GAPDHN failed to grow on gluconeogenic substrates under anaerobic conditions and ferment sugars because both: (i) lack of GAPDH and (ii) the irreversible oxidation of D-glyceraldehyde-3 phosphate (D-G3P) into 3-phosphoglycerate (3-PGA) in the presence of NADP, as evidenced by growth of the three clones harboring recombinant GAPDH (i.e., *E. Coli* W3CG transformed with plasmid harboring genes encoding 3 bacterial GAPDHs: pFV8, pFVA1 and pF61A) on gluconeogenic substrates (acetate and succinate as the sole carbon sources) under anaerobic conditions and fermentation of sugar as the wild type *E. Coli* W3CG (Valverde, page 156, col. 1, paragraph 2).

Furthermore, Valverde contemplates a strain of *E. Coli* engineered to contain both GAPDH and GAPN in the biotechnology of fermentative processes (page 158, col. 1, last paragraph), and in preferred embodiments, Valverde et al., teaches that the GAPDHN is able to function in its natural environments which can compete with advantage over the cytosolic NAD-dependent GAPDH due to its lower Km values for G3P and the pyridine nucleotide (p. 157, col. 1, last paragraph). Indeed, Valverde et al., discloses that GAPDHN has been found in some prokaryotic microorganisms including *Streptococcus* strains that lack the NADPH-generating enzymes of the oxidative pentose pathway and these strains simultaneously contain the NAD-depending phosphorylating GAPDH either

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to generate NADPH or ATP. Thus the art obviates a metabolically engineered *E. Coli* having both GAPDH and GAPDHN and thus the claimed first metabolic pathway of the invention (e.g., an intact GAPDH which produces NADH) would have been obvious.

At pages 9-13, Applicants essentially argue that: 1) the claimed yeast is further removed from Valverde *E. Coli* (or Boyd's *Streptococcus*) than are Vaeck's cyanobacteria, 2) Applicants are claiming more than the mere addition of a single heterologous gene to yeast as the invention claims creation of a new metabolic pathway that intend to interact with a native pathway, 3) the teachings of Nissen related to anaerobic fermentation in Yeast and the teachings of Valverde confined to *E. Coli* on the ability to metabolize sugar lost via deletion of GAPDH by introducing GAPN under aerobic conditions are very different fields as they are concerned with the metabolism of yeast and bacteria, respectively, 4) it would have been unknown whether GAPN could be expressed successfully and effectively in yeast, 5) it would have been unknown whether production of NADPH via expression and activity of GAPN would have any substantial effect on the level of NADH in yeast, 6) "the hope on which the Examiner's argument depends would be that avoiding one route to the production of NADH by using GAPN to produce NADPH instead would have a material effect", 7) the reference in Valverde to *Streptococcus* strains having both GAPDHN and GAPDH in the Boyd et al., publication of 1995 should have provided motivation to express both GAPDHN and GAPDH in yeast before Applicants' November 2002 priority date, 8) "it would have been completely unknown to what extent GAPN if expressed successfully in yeast would become engaged in glycolysis when competing with native yeast enzymes. It should be borne in mind that Valverde et al had deleted the *E. coli* GAPDH so had not even demonstrated that GAPN

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would have a material effect in *E. coli* in which the native glycolysis pathway had not been destroyed, let alone that it would be effective in yeast”, and 9) “the effect on the production of glycerol and ethanol in a yeast of this loss of ATP production would have been quite unknown. As seen in the diagram on page 157 of Valverde et al, ATP is required for consumption in the earlier stages of metabolism of glucose. Valverde et al had reported that their engineered *E. coli* had a decreased growth rate compared to wild type. A skilled reader would have good grounds for expecting that the hypothesized transformed yeast would also have decreased growth rates”. The above arguments have been fully considered but deemed unpersuasive.

Regarding 1) and 2), Applicants have not provided any probative evidence about how the claimed yeast is further removed from Valverde *E. Coli* (or Boyd’s *Streptococcus*) than are Vaeck’s cyanobacteria. The manipulation of previously known metabolic pathways in Yeast and *E. Coli* to improve respiration, fermentation, or enhancing a source of nutrients were well known in the art. Thus if GAPDH would oxidize $G3P \rightarrow 3\text{-PGA}$ in a recombinant *E. coli* with a mutated inactive GAPDH, it should be reasonably expected that the same GAPDH would oxidize $G3P \rightarrow 3\text{-PGA}$ in a recombinant yeast whether GAPDH is present or absent in the transformed yeast since G3P is the substrate of GAPDH and not GAPDH. In addition, engineering redox cofactors regeneration for improved fermentation in yeast was well known in the art at the time the invention was made as evidenced by the use of metabolically engineered *S. cerevisiae* overexpressing a heterologous recombinant yeast *Kluyveromyces lactis* *GDP1* gene encoding a NADP^+ -dependent glyceraldehyde 3-phosphate dehydrogenase

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(GAPDH) in a xylose-fermenting *S. cerevisiae* strain for enhanced ethanol production (Verho et al., Oct. 2003, pp. 5892-5897; Abstract, strain H2673, page 5893, Table 1).

Regarding 3), Applicant argues that Nissen related to anaerobic fermentation in Yeast and the teachings of Valverde are confined to *E. Coli* on the ability to metabolize sugar lost via deletion of GAPDH by introducing GAPN under aerobic conditions are very different fields as they are concerned with the metabolism of yeast and bacteria, respectively and the combined teachings would obviate the instant invention. In response to this argument, it is noted that a prior art reference must either be in the field of Applicant's endeavor or, if not, then be reasonably pertinent to the particular problem with which the Applicant was concerned, in order to be relied upon as a basis for rejection of the claimed invention. See *In re Oetiker*, 977 F.2d 1443, 24 USPQ2d 1443 (Fed. Cir. 1992). In this case, the disclosure of both Nissen and Valverde are concerned with metabolically engineering of microorganisms, e.g., yeast and *E. Coli* comprising levels of genes associated with phosphorylating and non phosphorylating branches of the glycolysis and alcohol fermentation, which is the field of Applicant's endeavor; therefore the combined disclosure of Nissen and Valverde is relevant to the instant invention.

Regarding 4) and 5), Nissen essentially discloses redirection of the flux of carbon from glycerol toward ethanol by modifying the ammonia assimilation pathway by reduced formation of surplus of NADH and ATP. Specifically, Nissen et al., describes a mutant *S. cerevisiae* wherein the normal NADPH-consuming synthesis of glutamate from ammonium and 2-oxoglutarate was substituted for a new pathway in which ATP and NADH were consumed. Valverde et al., essentially discloses a metabolic engineering glycolytic pathway with no energy yield by catalyzing the irreversible oxidation of D-

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G3P into 3-PGA in the presence of NADP⁺. Of note, GAPDHN is able to function in its natural environments which can compete with advantage over the cytosolic NAD-dependent GAPDH due to its lower K_m values for G3P and the pyridine nucleotide (Valverde, p. 157, col. 1, last paragraph). Thus Valverde et al., clearly discloses a new metabolic engineered redox pathway wherein cells are conceivable able to increase the formation of NADPH at the cost of NADH formation because both GAPDHN and GAPDH compete for the same G3P substrate. One of ordinary skill in the art would have been motivated to clone and express a recombinant GAPDHN in *S. cerevisiae* in an attempt to provide improved production of ethanol by reducing formation of surplus NADH by bypassing the ATP –reaction and by using NADP⁺ as a cofactor rather than NAD⁺, as a person with ordinary skill in the art has good reason to pursue the known options within his technical grasp.

Regarding 6), 7), 8) and 9), particularly Applicants' comments related to fact that Valverde et al., has not demonstrated that GAPN would have a material effect in *E. coli* in which the native glycolysis pathway had not been destroyed or alternatively in yeast, note that both yeast and *E. Coli* are fermenting microorganisms with a phosphorylating glycolytic pathway wherein G3P is functionally oxidized → 3-PGA. So if GAPDHN would functionally oxidized G3P → 3-PGA in a recombinant *E. coli* with a mutated inactive GAPDH, it should be reasonably expected that GAPDHN would functionally oxidized G3P → 3-PGA in a recombinant yeast with a mutated inactive GAPDH or active GAPDH for the same reason it oxidized G3P → 3-PGA in a recombinant *E. coli* with a mutated inactive GAPDH- it catalyzes the oxidation of G3P into 3-PGA.

Additionally, none of the references has to teach each and every claim limitation. If they

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did, this would have been anticipation and not an obviousness-type rejection. Therefore, Applicants' arguments that Valverde does not teach metabolically engineered yeast or that Nissen et al., does not disclose reducing formation of NADH and ATP by the enzymatic activity of a non-phosphorylating dehydrogenase are irrelevant.

References made of record in a PTO-892 Form to complete the record

Verho et al. *Appl Environ Microbiol.* 2003; pp. 5892-7.

Matsushika et al., *Applied Microbiology and Biotechnology*; 2009, pp. 37-53

Bro et al., *Metabolic Engineering*, 2006, pp. 102-111

Conclusion

Claims 1, 11-13 and 17-19 are rejected.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

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Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085.

The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Maria Leavitt/

Maria Leavitt
Primary Examiner, Art Unit 1633

Nissen et al., teaches a metabolically engineered *S. cerevisiae* for enhanced production of ethanol wherein reduced formation of surplus NADH and an increased consumption of ATP in biosynthesis results in decreased glycerol yield. Specifically, Nissen et al., describes a mutant *S. cerevisiae* wherein the normal NADPH-consuming synthesis of glutamate from ammonium and 2-oxoglutarate was substituted for a new pathway in

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which ATP and NADH were consumed. Nissen created a metabolically *S. cerevisiae* mutant in which *GLN1*, encoding glutamine cl as stated by argued